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Acute toxicity study and long term effect of daidzein on the thyroid gland of male Wistar rat

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Abstract- Daidzein is a plant extracted natural exogenous estrogen which can mimic the role of endogenous female hormone estrogen. It is a naturally occurring compound found exclusively in soybeans, moong beans, lentin, clover and other legumes. Daidzein was used for estimation of toxicity (LD_{50}) for short- term duration and its adverse effect on thyroid gland and its hormones in Wistar rat. Lethal $dose_{50}$ was estimated by log dose/ probit regression method. Five sets containing 10 rats each were taken and serially diluted doses were given orally with the help of intragastric gavage tube. The doses were 0, 50,100,150 and 300 mg/kg body weight (BW) for daidzein. The rats were observed for 4 days and then mortality was noted for further calculation. LD_{50} of the daidzein was found to be 150 mg/kg BW. $1/10^{th}$ of LD_{50} dose was introduced for sub-chronic 90 days treatment and its effect on thyroid hormones was observed after 90 continuous day's exposure. Saline water and ethanol (9:1v/v) were used as vehicle. The vehicle and daidzein solution were given intragastrically (0.5 ml/15 mg/kg BW) once a day for 90 continuous days. Serum TSH concentration was observed to be significantly ($p<0.001$) higher in daidzein treated animals in comparison to the control and sham - control animals. The levels of T_3 and T_4 were significantly lowered in daidzein treated rats. This result shows that the isoflavone, daidzein has adverse effect on thyroid gland and its function in Wistar rat. Thus, it can be suggested that daidzein administration affects the hormonal status of this animal.

Key words: Daidzein, LD_{50}, Toxicity, thyroid gland

INTRODUCTION

Daidzein is a natural endocrine disruptor belonging to class flavonoids and sub-class isoflavones that may interfere with the endogenous endocrine system and produce adverse developmental, reproductive and immune effects in animals. Structurally, daidzein closely resembles 17β-estradiol, and it binds to the estrogen receptors (ERs), the stronger affinity being for the ERβ isoform.[1] Thus they have potential to mimic and block sex hormones, adrenal function and even affect the thyroid gland function. Mimicking as a natural selective ER modulator daidzein exerts its estrogenic agonist and antagonist action as a tissue and dose dependent manner.[2] They are ubiquitously found in fruits, vegetables, grains, nuts, mostly abundant in soy beans and other legumes.[3,4] Approachability of soybean throughout popularized consumption in recent years due to their potential curative, preventative and nutritive value.[5,6] In addition to their positive effects, isoflavones have been shown to cause direct and indirect harmful effects on thyroid function of animals. Thyroid hormones regulate several metabolic processes and are known to play an important role in development and maturation of central nervous system, regulation of bone metabolism, cardiovascular system and

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normal growth in mammals. Studies on the relationship between soybean intake and thyroid function[7-10] suggest that consumption of soybeans and soy isoflavone daidzein, is goitrogenic and alters thyroid function. Estrogens exert several effects on thyroid follicular cells. They modulate their cell cycle regulation, proliferation and function thus potentially furnishing to the pathogenesis of thyroid hyperplasia and thyroid cancer. These effects are mediated by the binding to estrogen receptors.[11,12] However, the effects of soy isoflavones on thyroid function are controversial.[7-10] Several experimental studies have shown that administration of isoflavones at doses commonly found in human diets affects the thyroid gland function. Flavonoid rich diets have the potential to beneficial as well as adverse effects in humans.[13] This is related to hormonal activity, which is therefore relevant for the evaluation of associated risk to humans. Very few works are notable on the toxicity of LD_{50} of daidzein and its effects on mammals.

Therefore, the present study was aimed at determining the lethal dose toxicity and effect of daidzein after 90 days exposure on thyroid gland in Wistar rat.

RESEARCH DESIGN

The experiment was performed according to the guidelines accepted by the Ethics Committee, Ranchi University, Ranchi for investigations on animals.

Daidzein (7,4 dihydroxy isoflavones) is a naturally occurring exogenous endocrine disruptor present in a number of plants, mainly in soybeans and soy derived products. The toxicological study has been done on rats which are related to human beings and easy to handle in laboratory. Adult Wistar rats were selected from inbreed colony. Healthy adult rats of almost equal weight (130-150 g) body weight and size irrespective of sexes were selected randomly. The rats were housed in stainless -steel cages (435× 290×150 mm) and maintained in a room fully exposed to natural photoperiod, temperature and humidity. They were provided with food (soy free rat chow) and water *ad libitum*.

DETERMINATION OF LD_{50}

The LD_{50} was determined by log-dose/ probit regression live method.[14] Five sets containing 10 rats each were taken and serially diluted doses were given orally with the help of gavage tube. The doses administered were 0, 50, 100, 150 and 300 mg/kg BW for daidzein. The rats were observed for 4 days and then mortality was noted for further calculation. A graph has been plotted between empirical probit and log dose and then LD_{50} has been calculated with the help of regression live and computerized calculation.

SUB-CHRONIC TREATMENT

LD_{50} of the daidzein has been estimated (150 mg/kg bw) and $1/10^{th}$ of LD_{50} dose was introduced for sub-chronic 90 days treatment. Normal saline and ethanol (9:1v/v) were used as vehicle. All administrations were made intragastrically (0.5 ml/15mg/kg BW) once a day between 0900hrs-1000hrs for 90 continuous days. The animals were anaesthisized (di-ethyl ether) and their blood serum was collected and stored (-80^{o}C) until analysis.

ANALYSIS

Total tri-iodothyronine (T_3), thyroxine (T_4) and thyroid stimulating hormone (TSH) were measured by Chemiluminescence Immunoassay, CLIA.[15]

TABLES & GRAPHS

Table 1- Transformation of percentage to probit

%	0	1	2	3	4	5	6	7	8	9
0	---	2.67	2.95	3.12	3.25	3.36	3.45	3.52	3.59	3.66
10	3.72	3.77	3.82	3.87	3.92	3.96	4.01	4.05	4.08	4.12
20	4.10	4.19	4.23	4.26	4.29	4.33	4.36	4.39	4.42	4.45
30	4.48	4.50	4.53	4.56	4.59	4.61	4.64	4.67	4.69	4.72
40	4.75	4.77	4.80	4.82	4.85	4.87	4.90	4.92	4.95	4.97
50	5.00	5.03	5.05	5.08	5.10	5.13	5.15	5.18	5.20	5.23
60	5.25	5.28	5.31	5.33	5.36	5.39	5.41	5.44	5.47	5.50
70	5.52	5.55	5.58	5.61	5.64	5.67	5.71	5.74	5.77	5.81
80	5.84	5.88	5.92	5.95	5.99	6.04	6.08	6.13	6.18	6.23
90	6.28	6.34	6.41	6.48	6.55	6.64	6.75	6.88	7.05	7.33
---	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
99	7.33	7.37	7.41	7.46	7.51	7.58	7.65	7.75	7.88	8.09

Table 2- Influence of daidzein toxicity on male Wistar rat

Sl. No.	Concentration (mg/kg BW)	Concentration (ppm)	Number of animals		Percentage mortality	Log concentration	
			Exposed	Dead			
1.	300	6810	10	10	100	3.833	8.09
2.	150	1690	10	5	50	3.227	5.00
3.	100	750	10	3	30	2.875	4.48
4.	50	180	10	2	20	2.255	4.16
5.	0	0	10	-	-	-	-

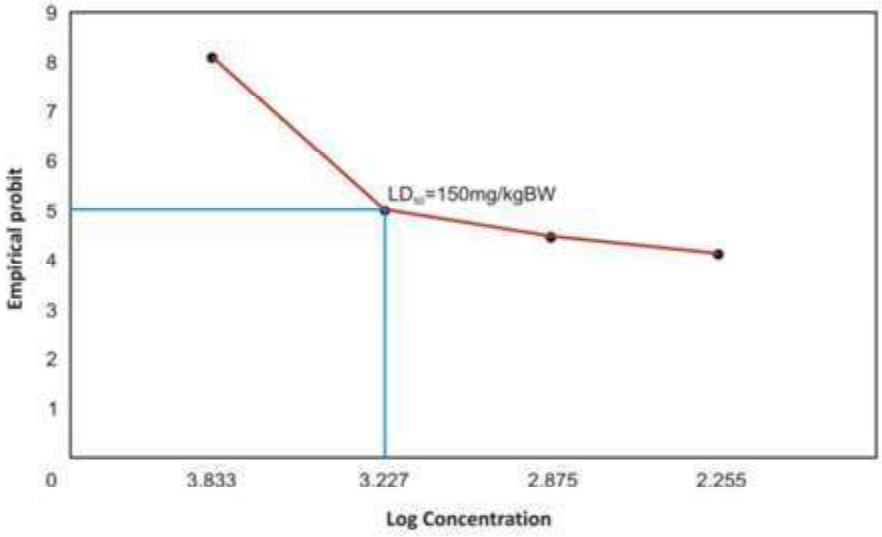

Figure 1- Plot of log concentration versus empirical probit from table 2 for calculation of LD_{50} of daidzein administered intragastrically.

Table 3- The influence of daidzein on serum thyroid hormones in mature male rats

Blood serum	Control	Sham-control	Treated
Thyroid stimulating hormone (TSH) µ/ml	0.85±0.02	0.86±0.01	2.83±0.02*
Triiodothryonine (T_3) ng/dl	132.0±0.04	131.9±0.05	77.0±0.05*
Thyroxine (T_4) µg/dl	7.30±0.04	7.31±0.08	4.60±0.06*

***Daidzein was dissolved in saline water: ethanol mixture (9:1 v/v; 0.5 ml/150g BW) and was administered intragastrically for 90 days. Values are means ± SEM for rats.**

RESULTS

In the present study the lethality of daidzein and mortality rate of rats were determined by introducing the daidzein orally. Daidzein in different concentrations/doses ranging from 50 mg/kg BW to 300 mg/kg BW were used. The animals exposed to different concentrations of daidzein, showed no mortality at 0 mg/kg BW groups, 20

percent mortality at 50 mg/kg BW, 30 percent mortality at 100 mg/kg BW, 50 percent mortality at 150mg/kg BW and 100 percent mortality at 300 mg/kg BW observed. The data was computed according to Probit Analysis Method[14] and LD_{50} value was determined.

Results for the effect of intragastric administration of daidzein at a dose of 15 mg/kg BW, for 90 continuous days on the serum levels of T_3, T_4 and TSH are presented in Table 3. It was observed that daidzein administration affected the hormonal status of the animal. Serum TSH concentration was significantly ($P<0.001$) higher in daidzein treated rat in comparison to the control and sham-control animals. The levels of serum T_3 and T_4 were lowered ($P<0.001$) in daidzein treated rats.

DISCUSSION

In the present investigation, the obtained LD_{50} value is 150 mg/kg BW.

The rats introduced to different concentration of daidzein, showed 50 percent mortality at 150 mg/kg BW. The probit mortality to the Wistar rat was calculated from percent mortality. When the probit mortality was plotted against log concentrations of daidzein, a straight line was obtained. The LD_{50} value obtained from this straight line graph is 150 mg/kg BW. The computation of percent mortality against different log concentration of the isoflavones yielded a sigmoid curve the LD_{50} value obtained from sigmoid curve was 150 mg/kg BW for 96 hours. The graphical representation of percent mortality versus log concentration and probit mortality versus log concentration of daidzein showed a typical sigmoid curve and straight line respectively which are in agreement with the principle of probit analysis.[14]

In the present study it was demonstrated that daidzein at the dose 15 mg/kg BW administered for ninety continuous days significantly increased the blood thyroid stimulating hormone (TSH) level in adult male rats. This was accompanied by a corresponding decrease in T_3 and T_4 concentrations. These present results provide evidence that intragastrically administered daidzein is able to influence TSH secretion from the pituitary gland. This may result in hormonal and metabolic disturbances. It has been reported that a subcutaneous injection of 10 mg/kg of daidzein can disturb the pituitary- thyroid axis, causing hypothyroidism in orchidectomized middle - aged rats.[16] Isoflavones such as daidzein and genistein have the ability to change thyroid cell events. Isoflavones can interfere with activity of an enzyme called thyroid peroxidase (TPO). TPO helps in attaching iodine to the amino acid tyrosine. This iodine tyrosine combination forms the basis for production of thyroid hormone. Daidzein and genistein act as suicide substrates for TPO by covalently binding to the active site. Thus, inhibition of TPO leads to a reduction of thyroid hormone levels, with a subsequent increment of TSH release. That, in turn, provides a strong growth stimulus to the thyroid gland. However, daidzein also affects the metabolism of thyroid hormones and iodide re-utilization by inhibition of sulfotransferase enzymes.[17]

In the present study the levels of thyroid stimulating hormone (TSH) in blood were significantly increased after treatment with isoflavone, daidzein. T_3 (tri-iodothyronine) and T_4 (thyroxine) levels showed an opposite effect and were lowered in treated animals as compared to the control and sham- control animals. Ferreira *et al.* (2002)[18] demonstrated that flavonoid daidzein inhibited 5'-iodothyronine deiodinase. In vivo experiments with the synthetic flavonoid EMD 21388, inhibits thyroid hormone binding to plasma transthyretin, showing a reduction of T3 content in tissues that express type II 5'-iodothyronine deiodinase.

In humans, Brahmbhatt *et al.* (2000)[19] showed that 530 children aged 6-15 years living in iodine-deficient areas of India and consuming large amount of isoflavones were found to be goitrous. This enormous rate of thyroid enlargement led to conclude, by these authors, that goiter was due to the combined effect of iodine deficiency and excess flavonoid in their diet.

Results obtained in our studying clearly indicate that daidzein significantly affects thyroid hormone levels in blood serum of the rat and suggest its involvement in thyroid action. Future research in this direction will provide a complete understanding of the isoflavone daidzein in thyroid function.

CONCLUSION

In the present study, the obtained LD_{50} value is 150 mg/kg BW. There is a shortage of information about the effect of pure isoflavones, such as daidzein, on thyroid safety in mammals. The results obtained in this experiment prove that 90 days sub-chronic intragastric administration of daidzein may change thyroid hormone function.

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